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TEST OF THE
PORTABLE WATER LABORATORY
ISOPOR TYPE, MODEL B, OF THE
AG CHEMICAL CORPORATION,
PASADENA, CALIFORNIA

TRANSLATION NO.

702

December 1962



U.S. ARMY BIOLOGICAL LABORATORIES FORT DETRICK, FREDERICK, MARYLAND

NO OTO

U. S. Army Biological

Laboratories: FD2-3742

JPRS: R-2928-D December 1962

TEST OF THE PORTABLE WATER LABORATORY ISOPOR TYPE, MODEL B, OF THE AG CHEMICAL CORPORATION, PASADENA, CALIFORNIA

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TEST OF THE PORTABLE WATER LABORATORY. ISOPOR TYPE, MODEL B, OF THE AG CHEMICAL CORPORATION, PASADENA, CALIFORNIA

[Following is the translation of a German-language document, entitled, in German, as above. As requested, a short summary has been provided by US JPRS, although a summary is included in the German text.]

Summary [by US JPRS]

The Portable Water Laboratory, Isopor Type, Model B, made by the AG Chemical Corporation, Pasadena, California, was found to be unsuitable for field use. The carrying case is poorly constructed and is too light in color for field work. Some essential items of equipment were broken or damaged during delivery transportation. The specified sterilization procedures are inadequate and under some conditions, damage the equipment. Spare or replacement parts are difficult to procure. In addition, certain auxiliary items of equipment, not provided by the manufacturer or deemed unnecessary by him were found to be essential for the proper functioning of the apparatus.

Federal Defense Department Testing Station for ABC Defense

Az.: B - 72 - 22 - 66 - 40/B 5/62

Munster, 28 March 1962 Tele.: MUNSTER 2831/583

TESTING ORDER

SUBJECT: Test of: Portable Water Laboratory, Isopor Type, Model B, of the AG Chemical Corporation. Pasadena. California. USA.

REF.: Testing Order PT III 1 - P 1082/59 C.

REPORTER: Agr. Eng. I. von Schonberg, TA.

ENCL.: 1 Short Manual.

TEST REPORT

Summary:

The testing apparatus is designed to perform on the spot, independently of any stationary laboratory, bacteriological analyses of water (including incubation), such as the degree of infection.

Important parts of the apparatus, however, are sensitive to transportation and weather conditions, jolting and knocking, as well also as to the classical sterilization methods. Without the latter, no incontestable results are obtained with this apparatus. All these facts that were brought out in the test are in contradiction to the statements concerning this in the prospectuses (Technical Bulletins No 14 - 16 of the AG Chemical Company).

The operation of the apparatus is quickly mastered and is performed without difficulty with the aid of the convenient, clearly expressed short manual.

The Isopor membrane filters (MF) and the appurtenant cardboard culture disks (NKS) [Nahrkartonscheiben] are suitable.

MF [membrane filters], made by the Gottingen Membran-filter-Gesellschaft, can be used in the testing apparatus. With regard to their size, specially manufactured ones are available. The original NKS of the Gottingen firm are also usable in combination with the Isopor MF.

Finally, it is determined that, because it is made of unsuitable material and due to its partially unsatisfactory technical performance and to its inconclusive analysis methods, the apparatus must be considered as not serviceable.

Task Report Director: Dr. Weitz RR Compiled by: von Schonberg, TA

Duty Assignment Director: Kramer, ORR Federal Defense Department Testing Station for ABC Defense

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ENCL.: 1 Short Manual.

A. Apparatus:

Portable Water Laboratory, "Isopor" Type, Model B.

Manufacturer: AG Chemical Corporation, Pasadena, California, USA.

B. Purpose of the test:

1. Examination of the technical performance.

- 2. Checking the working action described in the service instructions.
- 3. Test of the dry culture media (Isopor), supplied with the apparatus.
- 4. Test of dry culture media of the Gottingen Membranfiltergesellschaft in the "Isopor" apparatus.
- 5. Evaluation as to whether it is simple enough to manipulate so that it can be set up in the shortest possible time by trainees.
- C. Conduct of the testing:
 - 1. Examination of the technical performance.
 - 1.1 Carrying case
 - a. Overall impression of the carrying case.
 - b. Determination of the exact size.
- c. Determination of the weight without contents and with its contents ready for action.
- d. Evaluation of the carrying case with regard to transportation.
- e. Judgement of the carrying case with regard to setting up in the field.
 - 1.2 Evaluation of the contents with the help of the tasks performed with the apparatus:
 - a. Filter apparatus with sterilization tray.
 - b. Siphon bottle and tubes.
 - c. Hand vacuum pump and manometer.
 - d. Petri dish receptacle.
 - e. Petri dishes.
 - f. Frame with cover for the occasionally used Petri

liskes.

g. Alcohol burner.

- h. Plastic bottle for methanol.
- i. Glass bottle with a dose pipet for sterile water.
- j. Incubation flasks with a set of stands.
- k. Accessories: beaker, thermometer, tweezers, scissors, ballpoint pen, clamps, foil, bottles.
 - 1. Auxiliary and spare equipment.
- 2. Check of operation as provided for in the service instructions.

 The operation of the Isopor apparatus was performed systematically according to the appropriate instructions in the cover of the case.
- 3. Test of the dry culture media supplied with the apparatus.
 3.1 Examination of the cardboard culture disks (NKS) for sterility.

In this connection, the NKS were impregnated with sterile Koch's physiological salt solution and incubated without inoculation.

- 3.2 <u>Determination of the optimum dose of water</u> for impregnating the NKS.
- 3.3 <u>Incubation of inoculated Isopor membrane filters</u>, cultured from NKS taken from the Isopor Packs.
- 3.4 <u>Incubation of membrane filters from stock on hand</u> and likewise from NKS stock on hand stored for $1\frac{1}{2}$ years after manufacture.
- 4. Test of dry culture media from the Gottingen Membranfiltersesellschaft in the "Isopor" apparatus.
 - a. Gottingen membrane filter plates in the Isopor apparatus.
 - b. Isopor membrane filter plates incubated with Gottingen NKS.
- 5. Judgment as to whether it is simple enough to manipulate so that it can be set up in the shortest possible time by trainees.

This was performed just as in Point 2, above.

D. Results of the tests:

On Cl.1, a. The carrying case of the Isopor apparatus is made of synthetic material (see Figure 1). The lightweight material, fiber-plastic, called "fiber-glass" in the American test of the prospectus, is cloth fabric, scarcely 2 mm thick, stiffened with colorless synthetic resin, that, after fabrication, was covered over on both sides with a thin layer of rough, glossy mixed light green-white paste. It is inflammable, although not easily ignited. The green covering cracks when struck or scraped. In order to prevent scraping on the underside, four rubber stoppers are screwed on under the corners as feet.

The hinge found on the base of the front, the key lock, as well as the clasps and buckles for the leather handle are corrosion-resistant, as are the numerous rivets with which the holders are fastened inside the case to the case wall.

The holders are made of stainless, light metal; in the course of the test no fault could be found with them.

b. The dimensions correspond to those specified in the accompanying prospectus:

height 370 mm (with rubber feet)

width 430 mm depth 240 mm

- c. The weight of the carrying case with the stationary mounted parts (pump, manometer, sterilization tray, holders), without the rest of the contents, amounts to 5,820 grams; with the contents all ready to function (both incubation bottles filled, each with 750 ml of water), it is 13,750 grams.
- d. The complete apparatus is supposed to be carried comfortably by one person for short stretches by its wide, handy leather strap, provided that the only catch (lock) is intact and withstands the weight. Every time the apparatus is carried, above all in trucks and over uneven terrain, it is well to make sure of the following: Of the accessories made of glass, one incubation bottle and one Petri dish did not survive rail transportation as freight from Koblenz to Munster at the time of delivery. A supply of spare parts from the manufacturing firm, AG Chemical Corporation, Pasadena, California, USA, proved to be unavailable (lt. Nittlg. d. BWB Abtlg. PT III/l -1082/59/C of 28/10/59). Standard-sized German made articles do not fit the apparatus, and attempts at special manufacturing, for example Dewar receptacles in the incubation bottles, were unsuccessful. The damages originating from the delivery transportation make us conclude that the apparatus was not up to the strain of a shock test, and thus it was omitted during the testing, in view of the unavailability of a supply of spare parts that fit.

It is not possible to support the case by loading between packing cases or other apparatuses, since the rubber feet and the handle are in the way. Also, the apparatus must not be set up on edge, for the covers of the incubation bottles filled with water and of the Petri dish receptacles are put on only loosely.

The disadvantages that appeared here refute the statement in "Technical Bulletin No. 16" of October 1954, which states in the section under Point 3 pertaining to the carrying case: "The case is weatherproof and strong enough to permit transportation -- even under unusually rough conditions -- without damage to the contents."

e. Field installation means for the apparatus, setting it up to work under difficult conditions. The following is to be said about the carrying case:

The case is not sufficiently strong, and is unsuitable for transportation under average field conditions. Its single and, in addition, the weak lock is inadequate. Its rough surface is hand to clean, and is also too conspicuously colored for setting up in open terrain. It is not sufficiently sealed, and it does not protect the equipment within from dust and moisture, which is assumed for an apparatus intended for bacteriological purposes. Its inflammentality as disadvantageous, since work is performed within its walls each flame and easily inflammable reagents.

Syaluation of the contents (see Figure 2) with the help of the tasks performed with the apparatus.

The front of the carrying case, when let down, serves as while in use. During filtration, it must not lie either thank that it wobble. However, this is unavoidable in analyses

in the field, considering the light material of which it is made.

Filtration takes place on the filter stand, which is permanently mounted on the front of the carrying case. The filter apparatus is on the left side of the case on its sterilizer tray and, when not in use, is covered with the filter holder and is held with a spring.

The frit in the filter holder is a coke frit, on which the MF (membrane filter) is laid for filtration action. The filter holder is sterilizable both by boiling and in an autoclave; in field work, however, no other method can be used except the one specified in the operating instructions: formaldehyde fumigation. The sterilization effect of this method proved, however, to be insufficient. It will be commented on in detail under Point D, 2.

Flame sterilization tried out during the test destroys, with repeated use, the sealing material on the edges of the filter holder and definitely changes the structure of the coke frit.

The conical tapering underside of the filter holder gets rusty and is already distinctly roughened by corrosion. The body of the filter itself is strong, corrosion resistant and suitable for any kind of sterilization; however, the three springs on which the rotary locking rings are attached get rusty. The measurement graduations on the filter body (markings for 50, 100, 250, 500 ml) are perceptible only with difficulty.

The filter equipment is connected with the siphon bottle with a rubber tube.

Figure 2: Isopor Apparatus in Operation

Legend

- a. Filter equipment, shown here covered with the cover of one of the incubation bottles (j_1)
- b. Siphon bottle, tubes and tube clamp
- c. Hand vacuum pump, manometer behind it
- d. Petri dish holder without insert, with insert alongside on the left
- e. Petri dishes in the insert from (d)
- f. Mounting with cover for the occasionally used Petri dish
- g. Alcohol burner
- h. Plastic bottle with methanol
- i. Dose pipet in glass bottle with sterile distilled water
- j_{γ} . Incubation bottle, covered on the right, uncovered on the left
- j₂. Incubation stand, insert from the incubation bottle, Petri dishes for incubation stacked in it
- k. Beaker for taking samples and reheating water from the incubation bottles.
- 1. "Isopor Packs" containing several culture medium combinations (MF with NKS).

- b. The siphon bottle, made of strongglass, holds 1000 ml and is attached to the left rear corner of the carrying case by means of a spring-loaded holder. A piece of foam rubber serves as lower support. The bottle has a drain connection through which it can be emptied by means of a rubber tube, without having to remove it from its place. The drainage tube can be clamped to one of the walls of the case with a suitable clamping device. Cleaning and sterilization of this bottle is quite easy. The relatively wide neck of the bottle is closed with a rubber stopper, which has several holes for the insertion of accessory tubes. The bottle is connected with pump, manometer and filter equipment with rubber tubes that remain elastic even at low temperatures.
- c. Hand vacuum pump and manometer are stably and permanently mounted, the pump conveniently accessible and the manometer well visible.

The manometer is not an absolutely necessary accessory, for the creation of vacuum or the existence of leaks that prevent the creation of a vacuum, is perceptible through the difference in the stroke of the pump. The wooden handle of the pump was split due to twisting of the forced-in pump piston.

d. Both Petri dish receptacles are called, in the prospectus, "steel drums." They are very resistant to jolts and shocks. The plastic inserts in which the Petri dishes are stacked are firmly screwed on with the covers of the receptacles.

The receptacles corrode with wet sterilization (hot water, steam, superheated steam), although their use for hot water sterilization -- boiling for one-half hour -- is suggested in the short manual. Hot air sterilization must, however, be used with caution, on account of the plastic parts, and the temperature for this purpose may not exceed 120°C. With all the sterilization methods mentioned here, the plastic parts produced a strong, burning odor.

The receptacles are dust-proof when covered, if they are standing vertically. They open independently, however, if the apparatus is tilted, or if the receptacles themselves are upset. Then the inserts slide out with the Petri dishes stacked inside, and the Petri dishes get dirty or are damaged. One of the bottoms screwed onto the receptacles with rusty screws is not fitted on in a dust-tight manner.

Each receptacle with insert holds 12 Petri dishes that do not slip out of the inserts because of the sensible construction of the side supports.

e. The 24 Petri dishes are made of glass and are accordingly delicate. For incubation, they are not sealed with covers but rather with plastic film. After they have been sterilized, they are suitably kept in the above-mentioned inserts of the Petri dish receptacles with their openings turned down. This prevents soiling the uncovered topmost dish when the receptacle is opened.

f. For the rest of the Petri dishes that have already been prepared with NKS and MF, there is a mounting with a plastic cover on the front of the carrying case that serves as a work table. The Petri dishes stay in there, protected very well from any air, while the NKS is softening and before the MF is put on, and they can be sealed with the film. The cover is sterilizable by boiling; it melts if flame sterilization is used.

g. The alcohol burner, a small wick burner, that can also be operated with the methanol kept stored in the apparatus, burns with only a weak flame. The flame goes out if it is exposed to an air current in the open, and also it is not sufficiently intensive for a quick, reliable sterilization of the pipet, scissors and tweezers. The use of a soldering lamp would be suitable for the requirements of bacteriological work in the field, if the inflammability of the carrying case were not an obstacle. The reheating of water from the incubation bottles, in accordance with the incubation bottles, in accordance with the specified method, in the beaker on the filter stand with the wick burner indeed takes a long time with low outside temperatures, but it is feasible in principle.

h. A plastic bottle (100 ml) with a spray device holds the methanol for the sterilization method used in the apparatus.

i. A sterilizable bottle made of thick pyrex glass, capable of holding 250 ml, contains sterile water for soaking the culture media, and -- in a perforated stopper -- a reliably functioning dose pipet that is visible, according to the prospectus.

It is a question here of a ball pipet whose rubber ballon is compressed with a piston. The travel of the piston is shortened or lengthened by means of a screw device, whereby the amount drawn in by the pipet is controlled accordingly.

The amount of water that is dispensed is adjustable with this pipet to exactly 0.1 ml. Once it has been adjusted to the required measurement (here 2.3 ml), the pipet delivers each subsequent dose with an error of 10.1 ml. This error is admissible for softening the NKS.

j. The bright red incubation bottles (thermos bottles) are indeed not appropriately colored for use in a field installation.

They are the part of the Isopor apparatus that least withstands strain. Whereas one of the two bottles was already broken in rail transportation for delivery (see above), the second broke during the test.

The incubation procedure and the temperature lowering experiments connected with it (see the table referring thereto) were subsequently performed with Dewar vessels that corresponded approximately in size and contents to the original vessels.

The lateral supports of the incubation stand, made of synthetic material, and the inserts in the incubation bottles which are intended for receiving the Petri dishes and are packed together

with them in plastic bags and submerged in the incubation bottles, broke with normal usage. The breakage points were mostly over the holes drilled for screwing them on the bottom of the stand.

It is not possible to arrange the prepared Petri dishes in the narrow polyethylene bags without this stand and to insert them and submerge them in the bottles filled with water without this stand. Therefore, special importance must be laid on the quality of their material. It is expedient to fasten the incubation stand and bag together near the upper edge with tape or a rubber band that should be included with the apparatus.

k. The accessories (beaker, thermometer, tweezers, scissors, ballpoint pen, clamps, foil, bags) offer no cause for rejection.

The beaker is corrosion-resistant and fits well in the filter stand.

The thermometer, made of metal, is sturdy and measures approximately accurately in comparison with calibrated thermometers.

The tweezers with flat arms, the scissors and the ball-point pen are adequate.

The clamps for the open NKS bags and the supply of foil are suitable.

The supply of foil must be changed every two years at the latest: the tearing resistance of the foil also decreases with the lessened adhesiveness. Incubation under water is possible only as long as the foil adheres securily.

The bags (polyethylene) must be handled and preserved with care. Leaks caused by manufacturing faults or damages cast doubt on the incubation results of all the Petri dishes, which must be wrapped up watertight. It is advisable to test the bags for tightness by inflating before using.

1. This apparatus is also inadequate without auxiliary items and spare parts for the equipment, although the statement is made in the "Technical Bulletin No. 16," of October 1954, that:

"The Isopor Water Laboratory was so constructed that it satisfies the following requirements:

- l. It includes all the necessary accessories and all operating facilities for MF water examination (including incubating equipment) in one, single easily transportable piece.
- 2. It is independent of water, electricity, gas and the equipment of normal laboratories (autoclaves, ovens, etc.).
- 3. It eliminates any piece of equipment which is not easily manipulated and transported."

Auxiliary equipment for making dilutions are indeed superfluous with pure water examinations, but they are indispensable in raw water and waste water examinations (see also Point C. 2).

Accordingly, for the preparations of dilutions, the testing apparatus must accompanied by:

Reagent glasses, sterile, covered, with stands.

Measuring pipets, steile, with two cylinders.

Sterile distilled water or sterile Koch's physiological salt solution in bottles.

As spare parts for the apparatus, at least one each of the breakable parts of the apparatus must be available:

Incubation bottle with insert.

Siphon bottle.

Pipet tip for the dose pipet.

Bottle for the dose pipet.

Petri dishes.

Likewise, no provision is made in the apparatus for the adequate safe storage of <u>sufficient consumable material</u> (culture paper combinations, bags, foil, bands or rubber rings).

Not directly belonging to the apparatus, but important accessories for examinations are not taken into account in the carrying case: weatherproof matchbox, matches, notepad, rubber gloves, rags. For all this additional equipment, which does not belong to the standard equipment of the Isopor Water Laboratory, a special case must be provided to accompany the apparatus.

C. 2. Check of operation as provided for in the service instructions. An operating manual is glued to the cover of the carrying case in a conspicuous place. It is very readable, with fat type, and is provided with a transparent protective coating. A translation of this short manual is enclosed with this report. It is drawn up accurately. Preparations, filtration, incubation and the specified sterilization procedure can be performed quickly and accurately with the help of this manual. It is an extract from "Detailed Instructions for working with the Isopor Water Laboratory, Model B," published in Technical Bulletin No. 16, of October 1954. Technical Bulletin No. 16 is included with the apparatus by the manufacturer. In it equipment procedures and advantages are described very precisely; among other things, the concise instructions in the carrying case cover are amplified and treated very much in detail.

Some of the procedures used in the apparatus -- even if they are followed conscientiously with the help of the clear instructions -- seem to support the classical procedures:

The sterilization procedure (15 minutes of formaldehyde fumigation action that is produced in the apparatus by means of incomplete combustion of methanol) proves to be insufficient in test culturing Bact. E. coli and Bact. cereus. The small sealant coatings that are in the grooves of the filter holder do not withstand flaming the appratus. The apparatus begins to leak. In addition, the size of the pores in the coke frits could be changed with constantly repeated flaming. Even after prolonged operation of burning abundantly measured out methanol (20 seconds as opposed to 10 second in the manual) and after prolonged operation of formaldehyde fumigation (25 minutes against 15 minutes in the manual) this kind of sterilization is unreliable (see table referring thereto).

The specified procedure for preparing water samples, likewise speated in "Detailed Instructions for Working with the Isopor Water Laboratory, Model B." is to be rejected.

It states there under "Remarks on Water Samples, Paragraph B, Water Samples that contain more than one germ per milliliter, including all kinds of waste water:"

"In these cases, it becomes necessary to dilute the samples with sterile water. A simple, recommendable procedure for making the dilution -- particularly in the field where the requisite amount of sterile water is not available -- is the following: "A relatively clean water sample (100 - 1000 ml) is filtered into the siphon bottle through a membrane filter and kept there. This water can be considered -sterile.

"Before the analysis, the filter apparatus (already assembled with membrane) is then filled with 10 - 100 ml from the drain tube. The exact amount has no bearing.

"Approximately 1 ml of the water sample is drawn with a small pipet and is dropped into the water in the top part of the filter. For most samples of this type one gets, by counting the drops from the drop pipet, a dilution of sufficient accuracy (one drop = approximately 0.05 ml). Two drops of the water sample from the drop pipet, therefore, yield approximately 0.1 ml. In this way, the presence of 10,000 germs per ml (1,000 germs each M.F.) can be determined, without recourse to a line of dilutions in a series. It is to be noted that the same dilution water can be used repeatedly in the field, so that the requisite amount of dilution water is insignificant.

Ordinary drop pipets, normally found in trade, can be used (with a rubber balloon), one or more of which can be exposed to formaldehyde fumigation in the top part of the filter while sterilization is in progress. It is advisable to rinse with water from the siphon bottle, especially if a very dirty water sample (waste water) was in the drop pipet."

This procedure assumes the following:

Absolute sterility of the siphon bottle and of the rubber tubes, including the drain tube.

Absolute sealing between filter holder and filter top piece every time it is assembled. This is not always guaranteed.

Absolute germ impermeability of the filter plates. Occasional damage to the brittle, fragile membrane filter plates is, however, not to be excluded.

Absolutely successful sterilization by means of the specified formaldehyde fumigation procedure when sterilizing the pipets in the requisite manner. This procedure, however, is insufficient for the above-mentioned reasons, especially for pipets which stand only slight exposure to formaldehyde fumigation.

Moreover, it is absurd to start the first filtration with an elready slmost filled siphon bottle. Filtrations proceed better if the siphon bottle does not contain more than 500 ml.

For these reasons, the filtrate cannot be relied on as a sterile dilution reagent, and on the siphon bottle as a sterile space ressel, must as the sterilization of the drop pipet inside the torp piece of the filter cannot be relied on.

Therefore, it is necessary in preparing raw and waste water sendles, the take along dilution reagents and additional equipment are also Point 1.2, 1; "Aids and spare equipment").

The incubation method is to be recommended with reservations. The success of the incubation is dependent on a great number of sensitive factors, and especially on:

an incubation bottle with a well-insulating enclosure an insert with elastic side supports made of durable material

foil that adheres firmly undamaged bag

One defect in only one of these factors already throws doubt on the success of the incubation, and thereby on the results as a whole.

Temperature drop experiments could not be conducted on the original incubation bottles, because they already were defective at the beginning of the test (see Point 1.2, j) and substitutes could not be procured. They were performed with Dewar vessels that correspond somewhat to the shape and volume of the original incubation bottles:

Temperature Drop in Dewar Vessels

(Average Values)

Contents of the vessels: 850 ml of water

Constant exterior temperature	Starting temp. in vessel	<pre>\$ temp. drop, OC after 6 hours</pre>	<pre> temp. drop, oc after 12 hours </pre>
30	40	2.5	5.75
20	40	4.0	11.0
10	40	5.5	14.75
0	40	6.25	18.75
10	40	8.0	22.5

The drop in temperature increases very little with the number of Petri dishes inserted and the reduction in amount of water resulting therefrom. It is possible in the meanwhile to reheat the water in the beaker on the filter stand by means of the alcohol burner, provided with the equipment as suggested in the Manual. The incubation method could be perfected if strong incubation vessles were found, whose inside temperature is maintained constant, perhaps with the addition of a battery.

3. Test of the dry incubation media (Isopor), provided.

Only 12 each of the following types of Isopor cardboard culture disks (NKS) with their pertinent membrane filters (MF), 6 each, sterile, in polyethylene bags, in the so-called Isopor Packs, were enclosed with the apparatus:

General Type I for determining total germ count,

Endo's Type for determining germs of the coliaerogenes group.

These 24 NKS combinations were removed after delivery from their outermost wrapping that protected them from light and humidity. Their date of manufacture was not determinable. A greater number of cardboard culture disks was obtained through the firm of Otto Nordwald and Company, including some types not provided originally with the apparatus:

EMB Type (Koussin methylene blue) for differentiating between Bact. E. coli, on the one hand, and Bact. A. aerogenes, on the other. Bismuth sulfite Type for determining germs of the typhus-

paratyphus group.

3.1 Examination of the NKS for sterility:

When wrapping of the above-mentioned Isopor Packs was removed under sterile conditions, and were soaked in sterile Koch's physiological salt solution and incubated without inoculation, all the NKS combinations of all types proved to be sterile.

3.2 <u>Determination of the optimum water dose for soaking the</u> NKS:

The optimum water does is that specified in the Manual, namely 2.3 ml. Then one to two drops of excess water remain in the dish, which operate favorably to maintain humidity of the air in the dish during incubation.

3.3 <u>Incubation of inoculated Isopor membrane filters (MF)</u>
that were cultured from the Isopor packs.

The growth in the Isopor MF is satisfactory, with the

use of the pertinent Isopor NKS.

All germ colonies become sufficiently visible by means of using a General Type I redox indicator.

Filters stocked with germs that are incubated on the rest of the NKS types, also show the desired typical reaction after completion of incubation.

It must be taken into consideration, when the diluting material is chosen from raw and waste water, that the MF surface stocked with germs by filtration in the Isopor apparatus amounts only to 8.54 cm². The germs easily lie too compactly, and especially the ones falling on the same surface are no help at all in counting but 97 squares. With their 3 mm long, frequently unsharply delineated, edges they are too small and too numerous and consequently inconspicuous especially after incubation of the filters on dark a colored Endo's and EMB culture media. The filters, in a dry condition after use, are particularly brittle and fragile.

3.4 <u>Incubation of membrane filters in stock on hand</u> on similarly stored NKS at time intervals at the latest about $l_2^{\frac{1}{2}}$ years after manufacture.

The wholesalers indicated October-November 1960 as date of manufacture, so that these NKS were stored about $1\frac{1}{2}$ years by the end of the testing. After the time, the NKS combinations were still completely effective.

In <u>Technical Bulletin No 15</u> of the AG Chemical Company (manufacturing firm) the following is stated:

- ". . . Storageability exceeds two years under normal temperature and humidity conditions. Extreme care in manufacturing guarantees successful analyses."
- C. 4. Test of dry culture media from the Gottingen Membranfiltergesellschaft in the Isopor apparatus.
 - a. Gottingen membrane filter plates in the Isopor apparatus:

The original Isopor MF plates have a diameter of 47 mm.

The MF from the Gottingen Membranfilter Gesellschaft,
GmbH, for use in the Isopor apparatus are the "Special K 5 Membrane
Filter plates for Determination of Germ Content." They are manufactured with a diameter of 50 or 40 mm, and thus, as they come
commercially, they are too large or too small for use in the Isopor apparatus: The larger ones wrinkle and splinter when the filter top piece is screwed down, whereas the apparatus leaks when MF that are too small are used.

In accordance with arrangements made with the Gottingen MF-Gesellschaft, specially manufactured sizes are always available, by purchasing 1000 items, even without any advance in price worth mentioning.

b. Isopor membrane filter plates incubated on Gottingen NKS:
The size of the carboard culture disks is no consideration inasmuch as the original Gottingen 50 mm NKS fit easily in the Petri dishes of the Isopor apparatus. The dose pipet in the Isopor apparatus can be so adjusted that it dispenses, with one stroke, the 2.8 ml of water required to soften the Gottengen NKS.

Since incubation of the Isopor MF is also accomplished well, both technically and physiologically, on the somewhat larger Gottingen NKS, the use of original large Gottingen NKS in conjunction with Isopor MF would offer no difficulty. The Gottingen firm promised, however, with regard to the NKS, that they were also willing to stamp out any desired size — independently of the type of culture medium — if they were guaranteed a sufficient number of sales.

It is particularly to be pointed out in this connection that the Membranfilter-Gesellschaft GmnH firm was not informed, however, of the test of the Isopor apparatus in the Testing Station.

C.5 Judgment as to whether this kind of operation is simple enough for trainees to be able to set it up in the shortest possible time.

Operation of the apparatus is very simple in conjunction with the accurate short manual (In the cover of the apparatus), already described in paragraph D, 2. It can be learned by trainees in a short time and can be performed without assistance. The detailed explanations in the included prosperous, Technical Bulletin No 16, bring the understanding of the operation to the trainee.

If the results are still doubtful, then this is to be blamed on the uncertain procedures, likewise commented on in paragraph D, 2.

E. Summary.

The testing apparatus is designed to perform on the spot, independently of any stationary laboratory, bacterilogical analyses of water (including incubation) such as the degree of infection.

Important parts of the apparatus, however, are sensitive to transportation and weather conditions, jolting and knocking, as well also as to the classical sterilization methods. Without the latter, no incontestable results are obtained with this apparatus. All these facts that were brought out in the test are in contradiction to the statements concerning this in the prospectuses (Technical Bulletins Nos. 14 - 16 of the AG Chemical Company).

The operation of the apparatus is quickly mastered and is performed without difficulty with the aid of the convenient, clearly expressed short manual.

The Isopor membrane filters (MF) and the appurtenant cardboard culture disks (NKS) [Nahrkartonscheiben] are suitable.

MF [membrane filters] made by the Gottingen Membranfilter-Gesellschaft, can be used in the testing apparatus.

With regard to their size, a supply of necessarily specially manufactured ones is available. The original NKS of the Gottingen firm are also usable in combination with the Isopor MF.

Finally, it is determined that, because it is made of unsuitable material and due to its partially unsatisfactory technical performance and to its inconclusive analysis methods, the apparatus must be considered as not serviceable.

(v. Schonberg)

Dr. Weitz

Translation of the Manual, in the English language, that is placed, protected from moisture, in the cover of the portable Isopor Water Laboratory.

Before beginning:

Fill the incubation bottle with water at 39°C and close it tight.

Ensure that matches, cardboard culture disks and plastic foil are on hand in sufficient amount.

Put the alcohol lamp securely in place and fill its bottle, intended for that purpose, with sterile water and methanol. Sterilize the Petri dishes together with receptacles and the membrane filter apparatus. (Boiling for one-half hour suffices).

Conduct of the experiment or analysis.

- 1. Lift the membrane filter holder from the top piece of the filter and put it in the filter stand.
- 2. Light the alcohol lamp. Take a Petri dish out of the receptacle, put it in the dish stand and cover it with the plastic cover.
- 3. Take the automatic pipet out of the bottle of sterile water and distribute its contents on the Petri dish. Cover the dish again. Flame the tip of the pipet carefully and insert the pipet back in the bottle.
- 4. Take an "Isopor Pack," hold it with the side marked "Isopor" up and, with the scissors, cut the plastic bag and the cardboard along the marked line.
- 5. Flame the tweezers. With the side marked "Isopor" still up, press the edges of the packet together in order to spread out the opening and take out a membrane filter plate together with the protective sheet. Lay both on the membrane filter holder and then remove the protective sheet.
- 6. Place the top piece of the filter on the membrane filter holder; in addition, the rolls must lie over the openings on the edge of the holder. Hold the top piece firmly in one hand; with the other hand, turn the sealing ring in a clockwise direction until a marked resistance is felt.
- 7. Take the packet in the hand again (with the side marked "Iscpor" up), flame the tweezers and pick off one cardboard culture disk. Take care that the side of the packet that is upward continues to be held up. Hold the disk in a horizontal position precisely over the water in the dish. Let it drop like this onto the water. Cover the dish and clamp the cut-open edge of the Iscpor packet under the clamp.
- 8. Pour the water sample into the top piece of the filter. Pump until the vacuum meter indicates approximately 12 strokes.

- 9. After all the water has run through, remove the filter top piece and mark the membrane on the edge with the ballpoint pen. Flame the tweezers, take the membrane from the holder and then let it roll down from the edge onto the now moist culture disk, whereby air bubble formation is prevented.
- 10. Pull off a protective sheet of plastic foil and spread the foil over the dish. Then press the sides of the foil firmly around the edge and under the bottom of the dish. Invert the dish and insert it, upside down, in the incubation stand. Repeat all subsequent analyses in the same way from Point 3 on.
- ll. Pour half of the water out of the incubation bottle. Fasten the stand in such a way that the dishes are firmly secure, insert the stand in a polyethylene bag and put it in the incubation bottle. When one bottle is filled, take the other one. Occasionally check the water temperature. (Water can be warmed in the specimen beaker on the stand with the alcohol flame.)

When the analysis is finished:

- A. Extinguish the burner. Let the water run out of the siphon bottle and close the tube clamp again when the bottle is empty.
- B. Soak the piece of charcoal in the sterilization tray with methanol and light it. Bring the filter top piece to the place where it will be sterilized and cover it with the membrane filter holder. (Sterilization is finished after 15 minutes).